e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(5); 1322-1326

Original Research Article

Correlation between Serum Vitamin D and Soluble Urokinase Plasminogen Activator Receptor Levels in Patients with Extremely Drug-Resistant Tuberculosis at Vijayanagar Institute of Medical Sciences, Ballari, Karnataka.

D. Shanthala[1], V. Rajeshwari[2]

[1]Assistant Professor, Department of Biochemistry, Vijaynagar Institute of Medical Sciences, Ballari, Karnataka, India.

[2]Associate Professor, Department of Biochemistry, Vijaynagar Institute of Medical Sciences, Ballari, Karnataka, India.

Received: 25-02-2024 / Revised: 23-03-2024 / Accepted: 20-04-2024

Corresponding Author: Dr. D. Shanthala

Conflict of interest: Nil

Abstract:

Background: Tuberculosis (TB) is a critical global health issue, further complicated by drug-resistant forms like extremely drug-resistant tuberculosis (XDR-TB).Vitamin D deficiency is known to be associated with many infectious and metabolic disorders. Vitamin D related receptors have been reported in multiple tissues, where they play key role in immune system modulation. High prevalence of Vitamin D deficiency in pulmonary TB patients indicates that Vitamin D is a risk factor for development of XDR-TB. Previous studies have shown that soluble urokinase plasminogen activator receptor (suPAR) may be used as TB treatment efficacy marker. suPAR is cellular receptor for serine protease urokinase plasminogen activator. Bacterial endotoxins and cytokines of innate immune system stimulate secretion of urokinase plasminogen activator (uPA) in monocytes and neurophils. Serum suPAR levels are elevated when TB is active and decreases when patient responds positively to therapy.

Objective: To correlate between serum levels of vitamin D, known for its immune-regulating properties, and suPAR, an inflammation marker, in XDR-TB patients.

Design: The study was conducted in the Department of Biochemistry, VIMS, Ballari, Karnataka. The study subjects were selected from district TB hospital of VIMS, Ballari.

Controls: 36 age and sex matched healthy controls from the community selected.

Case: 36 cases diagnosed with XDR-TB from District TB hospital, VIMS, Ballari.

Methodology: Hb%, CBC, ESR, SGOT, SGPT, ALP, Blood Urea, Serum Creatinine, Serum Albumin, Serum Calcium, Serum Phosphorus were measured by standard procedures and Vitamin D levels by ELISA. Serum suPAR levels were measured by Quantitative sandwich enzyme immunoassay technique.

Results: A significant inverse correlation was found between serum Vitamin D and suPAR levels, indicating that lower vitamin D levels are associated with higher immune activation and inflammation.

Conclusion: The study suggests a link between vitamin D deficiency and increased inflammation in XDR-TB patients, highlighting the potential benefits of vitamin D monitoring and supplementation in managing this condition. Further research is needed to explore the therapeutic implications of vitamin D in TB.

Keywords: Vitamin D, suPAR, Extremely Drug-Resistant Tuberculosis, Immune Modulation.

Introduction

TB continues to be a significant global health concern, affecting millions of people and causing a substantial number of deaths. The rise of drug-resistant forms of TB, such as MDR and XDR TB, presents a major public health concern. The emergence of XDR-TB, with its resistance to multiple anti-TB drugs, has significantly complicated treatment regimens and led to reduced effectiveness, ultimately leading to worse patient outcomes and increased mortality rates [1].

There has been increasing attention towards the connection between vitamin D and the immune response to TB in recent years. Vitamin D is a fat-soluble vitamin that plays a crucial role in maintaining calcium and phosphorus balance and promoting healthy bones. However, it also has a significant impact on regulating the immune system[11]. The effects of Vitamin D are mediated by the presence of the Vitamin D receptor (VDR) on different immune cells, such as monocytes, macrophages, dendritic cells, and T and B

lymphocytes. These immune cells play a crucial role in protecting the body against Mycobacterium tuberculosis, the bacteria responsible for causing tuberculosis [2]

The role of vitamin D has been well characterized in the context of tuberculosis from a molecular standpoint. Activation of Toll-like receptors, a family of innate immune pattern recognition receptors, on human macrophages with M. tuberculosis-derived ligands, results in activation of the vitamin D pathway, including i) the conversion of 25-hydroxylvitamin D (25(OH)D) to 125-dihydroxy vitamin D (1,25(OH)2D), ii) activation of the VDR, and iii) antimicrobial activity against intracellular M tuberculosis infection. This provides a potential explanation for the association between the host vitamin D status with susceptibility to tuberculosis infection and disease [5].

Research has shown that maintaining sufficient levels of vitamin D can boost the immune system and lower the chances of contracting TB [3]. The antimicrobial activity of macrophages against Mycobacterium tuberculosis is enhanced by Vitamin D, which stimulates the production of antimicrobial peptides, cathelicidin and defensins. In addition, vitamin D plays a role in regulating the production of cytokines, which are molecules that can either promote or suppress inflammation. This balance is crucial for maintaining a healthy immune response and managing tuberculosis infection and inflammation [2 7].

A different approach biomarker that has gained attention in the context of TB is suPAR. suPAR is a soluble form of a receptor called suPAR, which can be found on different types of cells in the body, including immune cells and endothelial cells. Levels of suPAR in the blood can provide valuable insights into the immune system's activation state. These levels have been linked to the severity of diseases and can help predict prognosis in different infectious and inflammatory conditions, such as TB. Increased suPAR levels have been associated with active TB and unfavourable treatment results, indicating its potential as a prognostic indicator[67].

This study aims to explore the potential connection between vitamin D status and suPAR levels in patients diagnosed with XDR-TB. The focus is on understanding the immune modulatory effects of vitamin D and the prognostic value of suPAR in this specific patient population. Gaining a deeper understanding of this connection could offer valuable insights into the potential benefits of vitamin D supplementation as an additional treatment in enhancing immune function and improving treatment outcomes for patients with XDR-TB.

This study aims to investigate the vitamin D levels, its connection with the immune system, and the role of suPAR in prognostic indicator in TB. Through the exploration of these connections, our goal is to make a meaningful impact on the advancement of therapeutic strategies and enhance the outlook for patients fighting against XDR-TB.

Materials and Methods

This study was conducted in the Department of Biochemistry, VIMS, Ballari, Karnataka. This study was approved by the institutional ethical committee. The study subjects were selected from district TB hospital, VIMS, Ballari. An informed consent was obtained from all study subjects.

Controls

Randomly selected 36 age sex matched healthy controls from the community

Cases: 36 cases diagnosed with XDR-TB from District TB hospital, VIMS, Ballari

Inclusion Criteria: (1) Patients diagnosed as XDR-TB , based on culture and drug responsive testing, both males and females in the age group of 18-65 years. (2) Patients with treatment compliance.

Exclusion Criteria: TB patients with HIV/AIDS, Diabetes mellitus, cardiovascular disease, chronic renal failure, chronic Liver disease and neoplasms.

Diagnosis: Patients diagnosed as XDR-TB, based on clinical signs and X-Ray findings and history of treatment duration. A detailed history and Laboratory data were obtained from patient's clinical records. Their age, sex, height, weight, BMI, sputum smear data and chest X-Ray findings were noted.

Sample Collection: Under aseptic precaution, 4ml of blood was drawn from each of the study subjects, out of which 2ml was collected in EDTA bulb for estimation of HB, CBC, Erythrocyte Sedimentation Rate (ESR) by standard procedures. Another 2ml was collected in plain bulb and allowed to clot for 30 minutes at room temperature. The sample was centrifuged for 15 minutes at 100 *X g* and serum aliquots were stored at -20 c until further analysis for Vitamin D and suPAR. Blood Urea, Serum Creatinine, Serum Albumin, SGOT, SGPT, ALP, Serum Calcium, Phosphorus were measured in the serum sample immediately.

Procedure: Hb%, CBC, ESR, done in Fully automated hematology analyser as per the protocol. Blood Urea, Serum Creatinine, Serum Albumin, SGOT, SGPT, ALP, Serum Calcium, Phosphorus were measured in Erba 640 fully automated analyser. Vitamin D levels by chemi luminescent immune assay. Serum suPAR levels were measured by Quantitative sandwich enzyme immunoassay technique using R & D systems Human suPAR Quantikine ELISA kit.

Statistical Analysis: Statistical analysis was done by student t Test and one way ANOVA, and chi-square test to compare Vitamin D and suPAR levels. A p value of < 0.05 was considered as statistically significant.

Results

In this study, 36 patients with XDR-TB were analyzed. The demographic and clinical characteristics, along with the distribution of serum Vitamin D and suPAR levels, are detailed in Table 1. The findings show 72% of the patients were vitamin D deficient (<20 ng/mL), 25% had insufficient levels (20-29 ng/mL), and 3% had sufficient levels (≥30 ng/mL). The average serum Vitamin D level was 16.3 ± 5.2 ng/mL, while the mean serum suPAR level was 6.2 ± 3.1 ng/mL.

A significant inverse correlation was observed between serum vitamin D and suPAR levels ($r = -0.56$, $p < 0.001$), indicating that lower vitamin D levels corresponded with higher suPAR levels. Further analysis through multiple linear regression, adjusting for age, sex, weight, and duration of TB infection among other clinical variables, affirmed that serum 25(OH)D levels significantly predicted suPAR levels ($\beta = -0.49$, $p < 0.001$).

Comparative analysis between different patient subgroups based on the duration of TB infection revealed significant differences. Patients with longer TB durations (>12 months) displayed notably lower vitamin D levels (mean: 14.1 ± 4.7 ng/mL) compared to those with shorter durations (<12 months) (mean: 20.4 ± 6.1 ng/mL) ($p < 0.05$). Correspondingly, suPAR levels were higher in patients with longer TB durations (mean suPAR: 7.4 ± 3.2 ng/mL) versus those with shorter durations (mean suPAR: 4.5 ± 2.6 ng/mL) ($p < 0.05$).

Additional laboratory findings, including complete blood counts and liver and kidney function tests, are summarized in Table 4, which indicates that these are in normal range.

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	Value
Mean Age (years)	34.7 ± 12.5
Gender (Male/Female)	22/14
Mean Weight (kg)	38.2 ± 6.9
Mean Duration of TB (months)	13.8 ± 5.6
Symptoms (Fever/Cough)	100%
Pallor	100%

Table 2: Vitamin D and SUPAR Levels in Study Participants

Parameter	Value
Mean Serum 25(OH)D (ng/mL)	16.3 ± 5.2
Mean Serum SUPAR (ng/mL)	6.2 ± 3.1

Table 3: Comparison of Vitamin D and SUPAR Levels by TB Duration

Duration of TB Infection	Mean 25(OH)D (ng/mL)	Mean SUPAR (ng/mL)
< 12 months	20.4 ± 6.1	4.5 ± 2.6
> 12 months	14.1 ± 4.7	7.4 ± 3.2

Parameter	Mean ± SD
Hemoglobin (g/dL)	9.6 ± 1.2
Total Leukocyte Count (/mm³)	14500 ± 2500
ESR (mm/hr)	57 ± 12
SGOT (U/L)	45 ± 10
SGPT (U/L)	50 ± 12
Serum Calcium (mg/dL)	8.3 ± 0.5
Serum Phosphorus (mg/dL)	3.2 ± 0.6
Serum Albumin (g/dL)	3.7 ± 0.4

Discussion

This study investigated the correlation between vitamin D levels and suPAR levels in patients with extremely drug-resistant tuberculosis (XDR-TB). Findings demonstrated a significant inverse relationship, suggesting that lower vitamin D levels correlate with higher suPAR levels, thus providing insights into vitamin D's role in modulating immune responses and influencing disease severity in XDR-TB patients.

Vitamin D is recognized for its immune modulatory effects, enhancing the antimicrobial activity of macrophages against Mycobacterium tuberculosis and regulating cytokine production, which is crucial for a balanced immune response in TB control [8]. This study's results support the idea that sufficient vitamin D levels contribute to a more controlled immune response, potentially reducing inflammation and tissue damage [8,9].

suPAR, known as a biomarker of immune activation and inflammation, showed higher levels in patients with vitamin D deficiency, aligning with its association with disease severity and poor TB treatment outcomes [6,9,10]. This suggests that suPAR could be a useful prognostic marker in XDR-TB, indicating patients at risk of adverse outcomes.

The study also highlighted a troubling prevalence of vitamin D deficiency (72% of patients), emphasizing its significant role in immune function and TB outcomes. The inverse correlation between vitamin D and suPAR levels underlines the necessity of adequate vitamin D levels for modulating immune responses and possibly improving clinical outcomes in XDR-TB patients [11]

Clinically, these findings suggest the importance of routine vitamin D screening in TB patients, especially those with drug-resistant strains, and correcting deficiencies through supplementation to enhance immune response and treatment outcomes [12]. Monitoring suPAR levels could also provide critical prognostic information, guiding more personalized treatment strategies for patients with higher disease severity [13].

However, the study's cross-sectional design limits causality conclusions, and its findings may not be generalizable due to the small sample size. Additionally, factors like nutritional status, sun exposure, and genetic differences in vitamin D metabolism, which could affect results, were not considered. Future research should focus on longitudinal and randomized controlled studies to explore the causal relationships and effects of vitamin D supplementation on TB outcomes and investigate the mechanisms underlying the interaction between vitamin D and suPAR in TB pathogenesis and management [13,14,15].

Conclusion

This study shows a substantial inverse association between vitamin D and suPAR levels in XDR-TB patients, suggesting that vitamin D deficiency increases immunological activation and inflammation. These findings suggest vitamin D may modulate immunological responses and improve XDR-TB outcomes. Vitamin D screening, supplementation, and suPAR monitoring may improve XDR-TB therapy and prognosis. More study is needed to verify these findings and investigate vitamin D's TB treatment potential.

References

1. Parida SK, Axelsson-Robertson R, Rao MV, Singh N, Master I, Lutckii A, Keshavjee S, Andersson J, Zumla A, Maeurer M. Totally drug-resistant tuberculosis and adjunct therapies. Journal of internal medicine. 2015 Apr; 277(4):388-405.
2. Rathored J, Sharma SK, Singh B, Banavaliker JN, Sreenivas V, Srivastava AK, Mohan A, Sachan A, Harinarayan CV, Goswami R. Risk and outcome of multidrug-resistant tuberculosis: vitamin D receptor polymorphisms and serum 25 (OH) D. The International Journal of Tuberculosis and Lung Disease. 2012 Nov 1;1 6(11):1522-8.
3. Asani FF. Expression of vitamin D receptor (VDR) and VDR target genes in an African and Caucasian population: the impact of vitamin D

and mycobacterial elicitation. University of Johannesburg (South Africa); 2014.
4. Zhang J, Chen C, Yang J. Effectiveness of vitamin D supplementation on the outcome of pulmonary tuberculosis treatment in adults: a meta-analysis of randomized controlled trials. Chinese medical journal. 2019 Dec 20;132 (24):2950-9.
5. Zavala K. *Regulation of vitamin D metabolism during the immune response to mycobacterial infection* (Doctoral dissertation, UCLA).
6. Serum levels of soluble urokinase plasminogen activator receptor (suPAR) as a marker of tuberculosis treatment efficacy. Indian Journal of tuberculosis, vol64,no3/july2017. Indumati , V. Vijay, D. Krishnaswamy, V. Rajeshwari, A. Ramesh, D. Shantala, A. Shilpa
7. İLKİ AA. Tuberculosis and vitamin D. Marmara Medical Journal. 2014;27(2):85-.
8. Syal K, Chakraborty S, Bhattacharyya R, Banerjee D. Combined inhalation and oral supplementation of Vitamin A and Vitamin D: a possible prevention and therapy for tuberculosis. Medical hypotheses. 2015 Mar 1;84(3): 199-203.
9. Lange C, Abubakar I, Alffenaar JW, Bothamley G, Caminero JA, Carvalho AC, Chang KC, Codecasa L, Correia A, Crudu V, Davies P. Management of patients with multidrug-resistant/extensively drug-resistant tuberculosis in Europe: a TBNET consensus statement.
10. Sharma D, Sharma S, Sharma J. Potential strategies for the management of drug-resistant tuberculosis. Journal of Global Antimicrobial Resistance. 2020 Sep 1;22:210-4.
11. Baeke F, Van Etten E, Overbergh L, Mathieu C. Vitamin D3 and the immune system: maintaining the balance in health and disease. Nutrition research reviews. 2007 Jun;20(1):106-18.
12. Londt RS. Development of an Autologous Human Dendritic Cell Vaccine against Mycobacterium tuberculosis in Patients with Extensively Drug-Resistant Tuberculosis.
13. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immunomodulator. Immunology. 2011 Oct;134(2):12 3-39.
14. Edem VF, Ige O, Arinola OG. Plasma vitamins and essential trace elements in newly diagnosed pulmonary tuberculosis patients and at different durations of anti-tuberculosis chemotherapy. Egyptian Journal of Chest Diseases and Tuberculosis. 2015 Jul 1;64(3):675-9.
15. Singh A, Gupta AK, Singh S. Molecular mechanisms of drug resistance in Mycobacterium tuberculosis: Role of nanoparticles against multidrug-resistant tuberculosis (MDR-TB). NanoBioMedicine. 2020:285-314.